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Hyperhomocysteinaemia is an independent risk factor for peripheral arterial disease in a Chinese Han population

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Abstract

Introduction—Hyperhomocysteinaemia (HHcy) has long been suggested as a risk factor for atherosclerosis. However the association between HHcy and peripheral arterial disease (PAD) is still controversial. There is a lack of research on this topic in the Chinese population. This study aims to provide further results.

Methods—240 PAD patients and 240 control subjects were evaluated for both serum total homocysteine levels and ankle brachial indexes (ABIs). Multivariable logistic regression models were used to estimate the association between HHcy and the risk of developing PAD. Interaction and stratified analyses were conducted according to age, sex, smoking status, drinking status, and histories of chronic disease.

Results—The multivariate logistic analyses revealed that the risk of PAD was significantly associated with serum homocysteine levels. The interaction analysis showed no interactive role in the association between HHcy and PAD, indicating that homocysteine was associated with PAD independent of classical vascular risk factors.

Conclusion—In conclusion, HHcy is an independent risk factor for PAD in the Chinese Han population. A prospective and randomized clinical trial of homocysteine lowering therapy in the Chinese population is needed to assess the causal nature of the relationship.

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Conflicts of interest

There are no conflicts of interest associated with this study.

Author Contributions Statement

W.G. designed the study; J.L., G.S., H.L., and S.J. collected the data; D.R., J.L., and W.L. analysed the data; D.R., J.L., Y.L., H.Z., X.J., J.X., X.M., and X.L. analysed and interpreted the results; D.R., J.L., R.A.K., and D.N. wrote the article. All the authors have read and reviewed this manuscript.

Keywords

Peripheral arterial disease; Hyperhomocysteinaemia; Homocysteine; Case-control study

Introduction

Peripheral arterial disease (PAD) refers to the various diseases that affect noncardiac and nonintracranial arteries [1]. More than 200 million people worldwide are estimated to have PAD [2], and these statistics are expected to rise as the population grows and ages. Nevertheless, only 22% of PAD are symptomatic [3]. The most common cause of PAD is atherosclerosis, implying that the risk factors for PAD are similar to those for other atherosclerotic vascular diseases, with smoking and diabetes being the strongest two risk factors [2]. This means that more than 50% of the people who are diagnosed with PAD have also suffered from either coronary heart disease, cerebrovascular disease, or both [4]. Additionally, the incidence of adverse limb outcomes in PAD patients such as worsening of symptoms, peripheral revascularization and ischemic amputations was 26.2% in 4 years [5].

An elevated plasma homocysteine concentration is known as hyperhomocysteinaemia (HHcy). The association between HHcy and atherosclerosis was discovered in 1969 when McCully studied the vascular pathology of children with inherited disorders of homocysteine metabolism [6]. HHcy occurs in about 5 to 7 percent of the general population [7, 8], which is typically caused either by genetic defects in the enzymes involved in homocysteine metabolism or by nutritional deficiencies in vitamin cofactors (folate, vitamin B₁₂, and vitamin B₆) [9]. Several disease states also influence homocysteine metabolism, including chronic renal failure [10], hypothyroidism [8], pernicious anemia [11], and some types of tumor [12]. Moreover, HHcy has been suggested as a potent independent risk factor for arteriosclerosis for a long time [13].

Since McCully discovered that an elevated plasma homocysteine concentration could lead to atherosclerosis, abundant epidemiologic studies have been performed to validate the hypothesis in the western population [14–16]. In 1995, Boushey et al. [13] reviewed 27 studies on homocysteine and atherosclerotic disease and performed a meta-analysis of 5 studies revealing an association between elevated plasma homocysteine and PAD. In addition an updated meta-analysis [17] of 14 studies was performed in 2009 confirming the hypothesis that patients with PAD have significantly higher levels of homocysteine. Few similar studies [18, 19] were performed to investigate the association between HHcy and presence of PAD within the Chinese population, which compared to the western population are very different in their lifestyle, nutrition, and metabolism. In addition, the interactions of other cardiovascular risk factors such as age, smoking, and diabetes with HHcy in the development of PAD are still unknown. Therefore, this study aimed to assess the association between HHcy and PAD within the Chinese Han population, the main Chinese nationality with a population of up to 1.2 billion people. The influence of other classical cardiovascular risk factors on the association was also evaluated by using interaction and stratified analyses.

Methods

Study design and study population

In accordance with STROBE statement, we performed this case-control study with the purpose of estimating the potential relationship between HHcy and PAD. Data was collected between July 2011 and December 2012 in China. 240 patients diagnosed with PAD by photoplethysmography and computed tomography angiography (CTA) at the Vascular and Endovascular Department of the Chinese PLA General Hospital in Beijing and 240 control healthy subjects that underwent a medical examination in the same hospital were enrolled in this study. Participants were excluded based on the following criteria: (1) an ethnic origin other than Han; (2) a history of vitamin supplement intake; (3) a history of serum homocysteine related drug intake; (4) a history of serum homocysteine related disease (renal impairment, malignant tumor or hypothyroidism); (4) a history of mental disorder; (5) pregnancy; (6) an announcement of declining to participate. This study protocol was approved by the ethics committee of Chinese PLA General Hospital, and all subjects provided informed consent. All experiments were performed in accordance with relevant guidelines and protocols.

Data collection

Demographic characteristics, history of chronic disease, long term medication use, and lifestyle factors were recorded using a standardized questionnaire for subjects. Standard physical and biochemical examinations were completed in all participants. PAD was defined as a decreased ABI below 0.9 according to previous studies reporting over 90% sensitivity and specificity to detect PAD compared with angiography [20]. The subjects in the control group underwent a peripheral arterial photoplethysmographic[21] examination by two experienced specialists in order to exclude patients with PAD. Biochemical parameters were analyzed at the clinical laboratory of the Chinese PLA Hospital by using the Roche Modular chemistry analyzer (Roche Diagnostics, Basel, Switzerland). All blood samples were withdrawn after overnight fasting and were placed in freezer at 4°C immediately after phlebotomy. Centrifugation for separation of serum were carried out within 1 hour of collection and stored at -80°C until analysis.

Before undergoing peripheral arterial photoplethysmography, all recruited subjects were evaluated for the presence of risk factors for PAD. On the basis of self-reported histories, clinical examinations and biochemical tests, data on the following variables were recorded on a standard form: smoking habits, defined by a consumption of more than 100 cigarettes over a lifetime; drinking habits, defined by weekly white spirits consumption of more than 50 ml for a period over six months; hypertension, defined by a systolic blood pressure of more than 140 mm Hg or a diastolic blood pressure of more than 90 mm Hg (or both) on at least two occasions or by the use of hypotensive drugs; hyperlipidemia, defined by a fasting venous cholesterol level exceeding 5.7 mmol per liter, a fasting venous triglyceride level exceeding 1.7 mmol per liter, a fasting venous low-density lipoprotein (LDL) cholesterol level exceeding 3.4 mmol per liter, a fasting venous high-density lipoprotein (HDL) cholesterol level below 1.0 mmol per liter, or by the use of lipid-lowering drugs. Histories of diabetes, coronary artery disease (CAD), and ischemic stroke were self-reported. HHcy was

defined as a serum total homocysteine concentration exceeding 15 $\mu\text{mol/L}$, as determined based on the laboratory reference range.

Statistical analysis

Multiple inputs were used for missing data on smoking, drinking, LDL, HDL, hypertension, and diabetes. In total, 5 data sets were imputed, and results were pooled according to Rubin's rules [22]. Categorical variables were presented as frequencies and percentages, and continuous variables as means and standard deviations. The clinical characteristics of patients and control subjects were compared with the use of the Mann-Whitney test for continuous variables and the chi-square test for categorical variables. The association of HHcy and PAD was assessed with the use of multivariable logistic regression models. In another separate analysis, homocysteine was included as a continuous variable in the model. Unadjusted and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. We adjusted multivariable logistic regression models for age, sex, and other classical risk factors of vascular disease: smoking status, drinking status, coronary artery disease, ischemic stroke, diabetes, hypertension and hyperlipidemia. Interaction and stratified analyses were conducted according to age grouping (above and below 60 years), sex, smoking status, drinking status, and histories of chronic disease previously described. All of the analyses were performed with the statistical software packages R (<http://www.R-project.org>, The R Foundation) and EmpowerStats (<http://www.empowerstats.com>, X&Y Solution, Inc., Boston, MA).

Results

Characteristics of the subjects

There were 240 patients and 240 controls enrolled in this study. Table 1 shows the main characteristics of the study subjects as well as the prevalence of risk factors for PAD. Patients with PAD were slightly older (median age, 64 vs 59 years), smokers (119[49.6%] vs 46[19.2%]), had a diagnosis of hypertension (142[59.2%] vs 74[30.8%]), hyperlipidemia (168[70.0%] vs 123[51.2%]), diabetes (83[34.6%] vs 17[7.1%]), CAD (78[32.5%] vs 21[8.8%]), or ischemic stroke (62[25.8%] vs 13[5.4%]). Additionally, patients with PAD had a significantly higher (20.4 ± 11.5 vs 17.2 ± 8.7) serum total homocysteine than the control subjects.

HHcy and homocysteine level

HHcy was defined as serum total homocysteine levels $>15 \mu\text{mol/L}$ and was detected in 159 (66.2%) of the 240 PAD patients and in 121 (50.2%) of the 240 control subjects. The mean serum total homocysteine level in the PAD group was significantly higher than that in the control group ($20.4 \pm 11.5 \mu\text{mol/L}$ vs $17.2 \pm 8.7 \mu\text{mol/L}$; Figure 1).

Relationship between HHcy and PAD

The relationship between HHcy and PAD is presented in Table 2. The univariate logistic regression analysis revealed a significant association between HHcy and PAD (odds ratio [OR] = 1.93; 95% confidence interval [CI], 1.34–2.79). After adjusting for confounding variables, the risk of PAD was decreased in subjects with HHcy (OR = 1.63; 95% CI, 1.01–

2.63). When included as a continuous variable, homocysteine was also associated with an increased risk of PAD (adjusted OR per 1-unit increase in homocysteine, 1.03; 95% CI, 1.00–1.05).

The results of the stratified and interaction analyses of the association between HHcy and the risk of PAD are presented in Table 3 and Figure 2. The association between HHcy and PAD in the stratified analysis was consistent with that in the multivariable logistic regression analysis. The stratified analysis demonstrated a statistically significant association between HHcy and PAD in male subjects (adjusted OR, 1.5; 95% CI, 1.0–2.5), subjects younger than 60 years (adjusted OR, 1.9; 95% CI, 1.0–3.7), subjects without smoking habits (adjusted OR, 1.8; 95% CI, 1.0–3.2), without hypertension (adjusted OR, 2.0; 95% CI, 1.0–4.1), without diabetes (adjusted OR, 1.8; 95% CI, 1.0–3.1), without CAD (adjusted OR, 1.8; 95% CI, 1.0–3.1), without ischemic stroke (adjusted OR, 1.7; 95% CI, 1.0–2.9), and without hyperlipidemia (adjusted OR, 2.2; 95% CI, 1.0–5.1). The interaction analysis revealed no interactive role in the association between HHcy and PAD.

Discussion

In this case-control study, serum homocysteine levels were significantly associated with risk of PAD in the Chinese Han population. Subjects with HHcy had a 1.64-fold higher risk of PAD than those with normal serum total homocysteine levels. A 3% higher risk of PAD associated with a 1μmol/L increase in serum total homocysteine level further confirmed the relationship between homocysteine and PAD. Although participants with PAD were slightly older and had a greater prevalence of risk factors than controls, the association remained significant after adjustment for these risk factors. Moreover, we found no interactive role in the association between HHcy and PAD, suggesting that homocysteine is associated with the incidence of PAD independent of classical vascular risk factors.

An up to date meta-analysis revealed PAD to be significantly associated with elevated homocysteine levels which is consistent with our study result [17], however previous studies of PAD have yielded inconsistent outcomes. Ridker et al. [23] reported no association between homocysteine and PAD within the Physicians' Health Study (PHS, 1997–2007) cohort of male physicians. Aboyans et al. [24] and Taylor et al. [25] examined homocysteine levels and PAD progression, no association was discovered between homocysteine levels and PAD progression in both studies.

The decreased female sample size after stratification may have resulted in biases, but our study finding that homocysteine levels were associated with PAD in men is consistent with previous studies. Pradhan et al. [26] reported no association between homocysteine levels and the risk of PAD in the Women's Health Study (WHS, 1993–2004). Monica et al. [27] also reported no association between homocysteine levels and PAD within the Nurses' Health Study (NHS, 1990–2010) of female subjects. There may be gender differences in homocysteine metabolism as homocysteine levels are higher in men compared to women. One hypothesis has been postulated that these differences may be due to estradiol which lowers homocysteine levels in women [27,28]. More specifically, estrogens can lower plasma homocysteine levels by forming of estrogen-homocysteine conjugates [29]. Additionally,

catechol-O-methyltransferase (COMT) is responsible for both converting S-adenosyl-homocysteine to homocysteine and metabolizing catecholestrogens to methoxyestrogens and catabolize catecholamines [30,31]. Estrogens can also lower plasma homocysteine levels by competing with S-adenosyl-homocysteine for COMT. Other mechanisms contributing to gender differences of plasma homocysteine levels may exist simultaneously.

Female subjects are supposed to have a lower odds ratio for PAD since female subjects have a lower plasma homocysteine levels. However, the odds ratio for PAD of female subjects is significantly higher ($p=0.042$, not shown in results) than male subjects in figure 2. The power calculated by sample size and effect is 0.906, demonstrating that the female sample size meets the analysis requirement. We included homocysteine as a continuous variable in stratification analysis by gender, results showed that female subjects (OR=1.14; 95% CI, 1.04–1.25) have a higher risk of PAD than male subjects (OR=1.03; 95% CI, 1.00–1.05), females suffer a higher increased risk of PAD than males per 1-unit increase in homocysteine. Considering the results above, we hypothesize that females are more sensitive to plasma homocysteine. Fundamental experiment is needed to validate our hypothesis.

Although the association between HHcy and PAD in the stratified analysis was consistent with that in the multivariable logistic regression analysis, we found that it wasn't statistically significant in subjects older than 60 years, smokers and in subjects who were diagnosed with hypertension, diabetes, coronary artery disease, ischemic stroke or hyperlipidemia. CAD and ischemic stroke are also atherosclerotic vascular diseases which share similar risk factors with PAD; subjects with CAD and ischemic stroke are supposed to have higher homocysteine levels in both the case group and the control group. While aging, smoking, hypertension, diabetes, and hyperlipidemia are risk factors for PAD, subjects with these risk factors are also supposed to have higher homocysteine levels in both the case group and the control group. Consequently, residual confoundings were inevitable when we analyzed association of HHcy and prevalence of PAD in subjects with these factors. Additionally, the decreased sample size after stratification may also have resulted in biases.

Whether elevated homocysteine levels are a cause of atherosclerosis or a marker for atherosclerosis has yet to be confirmed [9]. The crude molecular mechanism of adverse vascular effects of homocyst(e)ine has been previously postulated. Oxidative metabolism of homocysteine to homocystine and homocysteine thiolactone would bring oxidative damage to vascular endothelial cells and increase the proliferation of vascular smooth-muscle cells [9]. B vitamins can lower homocysteine levels by promoting homocysteine metabolism [32]. Monica et al.[27] revealed an inverse association between higher folate intake (including supplements) and the risk of developing PAD in men. Moreover, a randomised trial showed that there was a significant improvement in ABI in participants who received either folic acid or 5-methyltetrahydrofolate compared with placebo [33]. The results of the China Stroke Primary Prevention Trial (CSPPT) also showed that participants in the enalaprol group have a higher risk of ischemic stroke than those in the enalapril-folic acid group [34]. However, the results of the Heart Outcomes Prevention Evaluation (HOPE) 2 study showed that daily administration of the combination of folic acid, vitamin B6, and vitamin B12 lowered homocysteine levels significantly but did not reduce the incidence of death from cardiovascular causes, myocardial infarction, and stroke during a mean follow-up period of

five years in a population with a history of vascular disease [35]. A meta-analysis of 8 randomized trials involving 37485 individuals found that although folic acid allocation reduced homocysteine levels by 25% on average, no significant effects on vascular outcomes was revealed during a median follow-up of 5 years[36]. Therefore, homocysteine could be a marker, but not a cause of vascular disease. Also homocysteine may play an important role in the initiation of atherosclerosis but does not affect its outcomes. Whether homocysteine contributes to the initiation and/or progression of atherosclerosis requires further investigation.

Further limitations of our study included: (1) recall bias, which couldn't be excluded because of the self-reported histories of chronic diseases, that affected the accuracy of estimation of disease prevalence; (2) the severity of PAD was neglected and only occurrence was recorded, thus the role of homocysteine in progression of PAD cannot be estimated(3) information of the MTHFR C677T polymorphism is lacking, therefore, we did not evaluate the roles of MTHFR C677T polymorphism in the association between HHcy and PAD; (4) vitamin B12 and folic acid levels were not measured, therefore, we can not evaluate the interaction between vitamins and homocysteine. These limitations should be considered in future studies.

In conclusion, HHcy is an independent risk factor for PAD in the Chinese Han population. Considering the limitations mentioned above, a prospective and randomized clinical trial of homocysteine lowering therapy in the Chinese population is needed.

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Abbreviations

HHcy	hyperhomocysteineamia
PAD	peripheral arterial disease
ABI	ankle brachial index
PLA	people's liberation army
LDL	low-density lipoprotein
HDL	high-density lipoprotein
CAD	coronary artery disease
OR	odds ratio

CI	confidence interval
PHS	Physicians' Health Study
WHS	Women's Health Study
NHS	Nurses' Health Study
5-MTHF	5-methyltetrahydrofolate
HOPE	Heart Outcomes Prevention Evaluation

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Figure 1.
Distribution of serum homocysteine levels in patients with PAD and controls.

Figure 2.
Odds ratios for peripheral arterial disease, according to baseline characteristics.

Table 1Main Characteristics of the Study Population.^a

Variable	Patients with PAD (N=240)	Control subjects (N=240)	P value
Age — yr	63.6±12.0	59.2±7.8	<0.001
Male sex — no.(%)	203(84.6)	195(80.0)	0.332
Smoking habits — no.(%)	119(49.6)	46(19.2)	<0.001
Drinking habits — no.(%)	63(26.2)	44(18.3)	0.037
Hypertension — no.(%)	142(59.2)	74(30.8)	<0.001
Hyperlipidemia — no.(%)	168(70.0)	123(51.2)	<0.001
Diabetes — no.(%)	83(34.6)	17(7.1)	<0.001
CAD — no.(%)	78(32.5)	21(8.8)	<0.001
Ischemic stroke— no.(%)	62(25.8)	13(5.4)	<0.001
tHcy — μmol/L	20.4±11.5	17.2±8.7	<0.001

^aPlus-minus values are means ±SD. P values were calculated by comparing characteristics between two groups.

Table 2

Multivariable logistic regression models evaluating the association between tHcy and PAD.

Variable	No.(%) of study participants		OR(95%CI)	
	Cases	Controls	Crude	Adjusted ^a
tHcy,1μmol/L	240	240	1.03 (1.01, 1.05)	1.03 (1.00, 1.05)
tHcy<15μmol/L	81(33.8)	119(49.6)	Ref	Ref
tHcy ≥15μmol/L	159(66.2)	121(50.2)	1.93 (1.34, 2.79)	1.63 (1.01, 2.63)

^a Adjusted for Age, Sex, Smoking status, Drinking status, Coronary Artery Disease, Ischemic stroke, Diabetes, Hypertension and Hyperlipidemia.

Table 3

Association between hyperhomocystenaemia (HHcy) and PAD according to baseline characteristics.

Subgroup	No.(%) of study participants				OR(95%CI)		P Value for interaction
	Cases(N=240)		Controls(N=240)		Crude	Adjusted ^a	
	HHcy	nHcy	HHcy	nHcy			
Age							0.25
<60	56	33	59	74	2.1 (1.2, 3.7)	1.9(1.0, 3.7)	
60	103	48	62	45	1.6 (0.9, 2.7)	1.3(0.6, 2.7)	
Sex							0.55
male	139	64	112	83	1.6 (1.1, 2.4)	1.5 (1.0, 2.5)	
female	20	17	9	36	4.7 (1.8, 12.5)	2.3 (0.6, 8.6)	
Smoking habits							0.91
No	75	46	92	102	1.8(1.1, 2.9)	1.8 (1.0, 3.2)	
Yes	84	35	29	17	1.4(0.7, 2.9)	1.4 (0.6, 3.2)	
Drinking habits							0.88
No	115	62	97	99	1.9(1.2, 2.9)	1.6 (0.9, 2.9)	
Yes	44	19	24	20	1.9(0.9, 4.3)	1.8 (0.6, 5.1)	
Hypertension							0.73
No	62	36	78	88	1.9 (1.2, 3.2)	2.0 (1.0, 4.1)	
Yes	97	45	43	31	1.6 (0.9, 2.8)	1.6 (0.8, 3.3)	
Diabetes							0.27
No	115	42	113	110	2.7 (1.7, 4.1)	1.8 (1.0, 3.1)	
Yes	44	39	8	9	1.3 (0.4, 3.6)	0.8 (0.2, 2.6)	
CAD							0.52
No	108	54	108	111	2.1 (1.3, 3.1)	1.8 (1.0, 3.1)	
Yes	51	27	13	8	1.2 (0.4, 3.1)	1.1 (0.3, 3.6)	
Ischemic stroke							0.79
No	117	61	114	113	1.9 (1.3, 2.8)	1.7 (1.0, 2.9)	
Yes	42	20	7	6	1.8 (0.5, 6.1)	2.0 (0.4, 11.4)	
Hyperlipidaemia							0.31
No	44	28	51	66	2.0 (1.1, 3.7)	2.2 (1.0, 5.1)	

Subgroup	No.(%) of study participants				OR(95%CI)		P Value for interaction
	HHcy	nHcy	HHcy	nHcy	Crude	Adjusted ^a	
Cases(N=240)	115	53	70	53	1.6 (1.0, 2.7)	1.4 (0.8, 2.5)	
Controls(N=240)							
HHcy							
nHcy							
Yes							

^aEach stratification adjusted for all the factors(Age, Sex, Smoking status, Drinking status, Coronary Artery Disease, Ischemic stroke, Diabetes, Hypertension and Hyperlipidemia) except the stratification factor itself.